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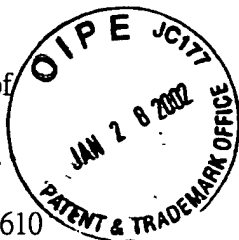
In re Patent Application of

Simon C. BURTON et al.

Application No.: 08/468,610

Filed: June 6, 1995

For: CHROMATOGRAPHIC RESINS AND
METHODS FOR USING SAME



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) Group Art Unit: 1651
)
) Examiner: Jon P. Weber, Ph.D.
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DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Nathaniel T. BECKER, do hereby declare:

1. THAT, I have received a Bachelor of Science Degree in chemical Engineering from Stanford University in 1983, and a Master of Science Degree from the University of California, Davis in 1984.

2. THAT, I am an employee of Genencor International, Inc. (hereinafter "Genencor"), where I have worked since 1985. I am a biochemical engineer by training and am currently the Group Leader for the Delivery Systems group at Genencor. I spent more than eight of my seventeen years at Genencor developing separation and purification processes for the production of enzymes and proteins at industrial scale. This includes extensive experience developing and inventing processes and techniques based on the following technologies: centrifugation, filtration, extraction, chromatography (ion exchange, hydrophobic, affinity), cross-flow membrane filtration,

Considered
[Signature]
13 June 02

crystallization and precipitation. I have numerous patent applications and several issued patents in the areas of protein crystallization and chromatography. Most specifically, I have carried out and directed experimental work involving the separation of enzymes and proteins using ion exchange chromatography and other forms of chromatography, which requires a knowledge of how to prepare, adsorb and elute proteins from ion exchange columns.

3. THAT, a copy of my Curriculum Vitae is attached hereto as Appendix A.

4. THAT, I am one of the inventors of the subject matter disclosed and claimed in the above-referenced application, and I have reviewed and am familiar with the contents of U.S. Patent Application Serial No. 08/468,610 (hereinafter "'610 patent application") including currently pending claims 1-5 and 7-23.

5. THAT, the invention in the '610 patent application relates to complexes of chromatographic resins with proteins and peptides. In particular, the chromatographic resins are useful for the binding of a target protein or peptide from an aqueous medium. Central to the claimed invention is the use of an electrostatically uncharged resin at the pH where the target protein or peptide is bound to the resin which has a pH in the range of from 5 to 9. In addition, the resin is selected such that it contains an electrostatic charge at the pH where the protein or peptide is desorbed from the resin, wherein the desorption occurs by a change in the pH from the binding pH.

6. THAT, I have reviewed and am familiar with the Office Action dated September 28, 2001. I have also reviewed and am familiar with the Examiner's rejection of the claims alleging that such claims are purportedly anticipated by Boardman et al., *Nature*, 171:208-210 (1953) (hereinafter "Boardman").

7. THAT, I have reviewed and am familiar with the product literature regarding the synthetic cation exchange resin, Amberlite IRC-50, provided by the manufacturer, Rohm and Haas Co. (2000) (hereinafter "Rohm and Haas"). A copy of the Rohm and Haas product literature is attached hereto as Appendix B.

8. THAT, I have reviewed and am familiar with the Boardman reference. In relation to this reference, the September 28, 2001, Office Action contains the following statement:

"At a low pH the cation exchange media is uncharged and binds the proteins. As the pH is raised, the protein is eluted. Figure 1(a) illustrates the technique with cytochrome C on Amberlite IRC-50 [a cross-linked poly(methacrylic acid) with a capacity of 10 Meq/g]. At a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged."

9. THAT, in my opinion the above statement regarding the Amberlite IRC-50 resin is inaccurate. By definition, and as is recognized by those skilled in the art, Amberlite IRC-50 is a weakly acidic cation exchange resin based on macroreticular methacrylic acid-divinylbenzene chemistry. It has a pK value of 6.1, meaning that it is still 50% charged at pH 6.1. The charged moiety on the resin is a carboxylic acid group within the methacrylic acid functionality. Given that the resin is weakly acidic, it retains a partial charge at pH 5, and becomes fully protonated (neutralized) only at a pH of between 2.5 and 4.0, depending on the buffer salts present. This is clearly demonstrated in the product literature provided by the manufacturer, Rohm and Haas. According to Rohm and Haas, the point of zero net charge is equivalent to the pH at which zero milliequivalents of base (KOH) have been applied to the resin, which is represented by where the titration curves intersect the y-axis (pH) at zero on the x-axis (mEq KOH) (see Figure 3). This will vary slightly depending on the buffer salts, but, at most, is pH 4.0 for pure water. Thus, it is

my opinion that the Amberlite IRC-50 cation exchange resin in Boardman remains charged at the pH where it binds the protein.

10. THAT, in my opinion those skilled in the art would recognize that no basis is seen in the Office Action or in Boardman to conclude that Boardman teaches binding a protein to an uncharged resin in the range of pH 5 to 9. Accordingly, the Amberlite IRC-50 cation exchange resin of Boardman fails to disclose the claimed invention. Thus, the Boardman reference neither anticipates nor renders obvious the claimed invention.

11. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 1-25-02

Signed: Nathaniel T. Becker

Nathaniel T. Becker

APPENDIX A

Nathaniel Todd Becker
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Hillsborough, CA 94010
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EDUCATION

Brown University, A.B., Philosophy (magna cum laude), 1979
Stanford University, B.S., Chemical Engineering, 1983 and M.A., Philosophy, 1983
University of California, Davis, M.S., 1984

EMPLOYMENT HISTORY

Genentech, Inc., South San Francisco, Ca.

1983- 1984 Biochemical engineering intern

Genencor International, Inc., Palo Alto, Ca.

1985-1989 Advanced from research associate to scientist in the area of enzyme recovery and purification. These responsibilities included technical responsibility for developing and scaling up downstream processes based on centrifugation, filtration, membrane separations, chromatography and crystallization

1989-2001 Advanced from scientist to staff scientist, currently Group Leader for the Delivery Systems group. Responsibilities included development and commercial deployment of liquid and solid formulation technologies, including stabilization, granulation and coating, and controlled release technology. This has included project leader responsibilities for over ten new products, and inventorship on more than a dozen patents and patent applications in the area of purification, formulation and granulation of enzymes.

PUBLICATIONS (Selected)

- Becker, T., Park, G. and Gaertner, A.L. (1997). Formulation of detergent enzymes. In: Van Ee, J.H., Misser, O. and Baas, E. (eds). *Enzymes in Detergency*. Marcel Dekker, New York, pp. 299-325.
- Becker, T. (1995). Separation and Purification Processes for the Recovery of Industrial Enzymes, Ch. 14 in *Bioseparation Processes in Foods*, R.K. Singh and S.S. H. Rizvi, eds., Marcel Dekker, New York, 1995, pp. 427-445.

APPENDIX B



AMBERLITE[®] IRC50

Synthetic Cation Exchange Resin

PRODUCT DATASHEET

AMBERLITE IRC50 is a synthetic cation exchange resin produced in the form of white, opaque beads. Its unusually high exchange capacity is derived from carboxylic acid groups. Supplied in the hydrogen or "free-acid" form, AMBERLITE IRC50 can be converted readily to the sodium salt by treatment with a solution of sodium hydroxide. In the sodium form, the resin undergoes reaction typical of the salt of a weak acid and strong base.

Because of its selectivity for the hydrogen ion, any adsorbed cation can be desorbed easily with

a regeneration efficiency approaching 100% by treatment with dilute mineral acid. The carboxylic functionality and exchange selectivities of AMBERLITE IRC50 lead to immediate consideration of this ion exchange resin in a variety of applications such as the neutralisation of strong bases; the recovery of metallic ions; the isolation and concentration of antibiotics; basic amino acids, enzymes and peptides.

PROPERTIES

Physical form	White opaque beads
Ionic form as shipped	H ⁺
Total exchange capacity ⁽¹⁾	≥3.0 eq/L (H ⁺ form)
Moisture holding capacity ⁽¹⁾	43 to 53% (H ⁺ form)
Shipping weight	660 g/L
Particle size	
Harmonic mean size ⁽¹⁾	280-700 μm
Uniformity coefficient ⁽¹⁾	≤2.0
Fines content ⁽¹⁾	<0.300 mm: 8.0% max
Coarse beads ⁽¹⁾	>1.180 mm: 5.0% max
Maximum reversible swelling	H ⁺ → Na ⁺ : 100% H ⁺ → Ca ²⁺ : 40%

⁽¹⁾ Contractual value

SUGGESTED OPERATING CONDITIONS

Maximum operating temperature	100°C
Minimum bed depth	600 mm
Service flow rate	8 to 16 BV*/h
Regenerants	HCl or H ₂ SO ₄
Concentration (%)	2 to 5 0.5 to 0.7
Flow rate (BV/h)	2 to 8 15 to 40
Level	See text
Rinse water requirements	4 to 7 BV
Service flow rate	8 to 16 BV*/h

*BV (Bed Volume) = 1 m³ solution per m³ resin

PHYSICAL STABILITY

Effect of Temperature

The rate of exchange and the affinity for hydrogen (H^+) increase as operating temperatures are elevated.

Attrition

Extended field experience has shown that AMBERLITE IRC50 has excellent attrition resistance.

CHEMICAL STABILITY

AMBERLITE IRC50 is stable in the presence of strong alkalis and acids, aliphatic and aromatic solvents. On prolonged contact with certain organic solvents, the resin swells to some extent, but no disintegration of the exchanger bead has been observed.

OPERATIONAL CHARACTERISTICS

Pressure Drop

The approximate drop in pressure to be expected for each metre of bed depth of AMBERLITE IRC50 in normal downflow operation at various flow rates and temperatures is indicated by the data in Figure 1.

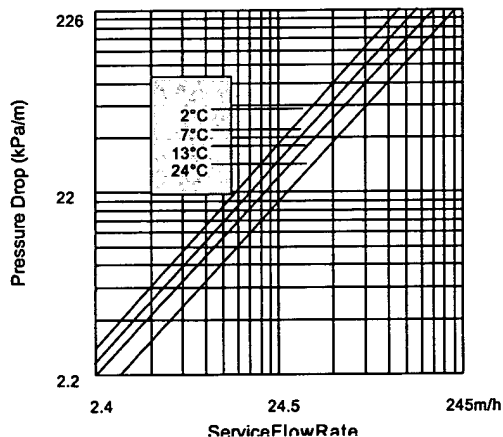


Figure 1

Hydraulic Expansion

To ensure proper cleaning and hydraulic classification of AMBERLITE IRC50 after each operational cycle, the bed of resin should be backwashed with water for about ten minutes at a flow rate sufficient to effect a minimum of 50% expansion in bed volume.

The hydraulic expansion of the bed during backwashing operations is reported as a function of the flow rate at various temperatures in Figures 2a and 2b.

Values for the calcium and sodium forms are used in the example.

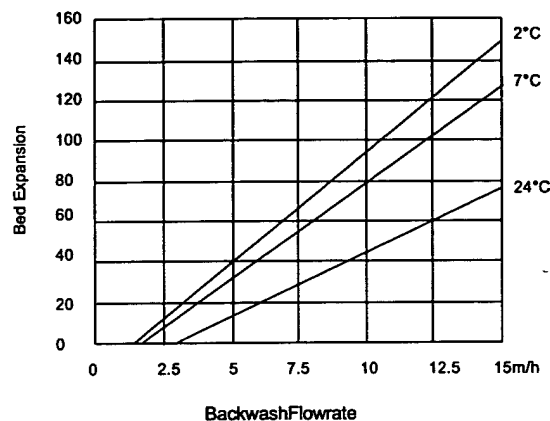


Figure 2a: Hydraulic Expansion (Ca)

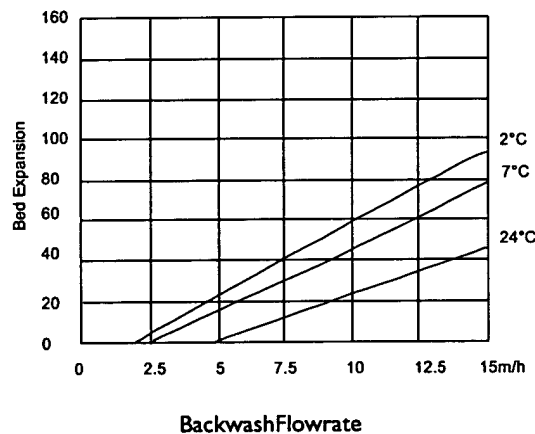


Figure 2b: Hydraulic Expansion (Na)

EXCHANGE CAPACITY

The total exchange capacity of AMBERLITE IRC50 is attainable only at high pH values. In strongly alkaline media, it is possible to utilise all of the carboxylic acid groups calculated to be present in the resin matrix.

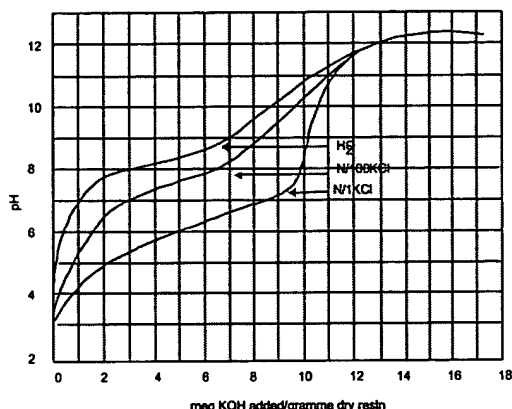


Figure 3: Titration curves

ORGANIC COMPOUNDS

The buffering capacity and acid-elution efficiency of AMBERLITE IRC50 have been demonstrated by applying the exchanger to the adsorption and recovery of basic amino acids (arginine, histidine and lysine); alkaloids (nicotine and quinine); vitamins (thiamine); and miscellaneous bases such as adenine, pyridine, hydrazine and ammonia.

BASIC AMINO ACID ADSORPTION

Amino Acid	Column Pre-Treatment	Leakage %	Capacity g/L
Histidine	H-resin	25	very low
Lysine	H-resin	25	very low
Arginine	H-resin	25	very low
Histidine	Column buffered—pH 5.75	25	very low
Histidine	Column buffered—pH 5.30	10	very low
Histidine	Column buffered—pH 5.0	5-10	18
Histidine	Column buffered—pH 4.7	2-10	35
Lysine	Column buffered—pH 7.0	0-3	120
Lysine	Na-resin	Ca-100	very low
Arginine	Column buffered—pH 7.0	0-2	150
Arginine	Na-resin	30	—
Leucine	pH 4.70 buffer	100	nil
Glutamic Acid	pH 4.70 buffer	100	nil

Basic amino acids separations with AMBERLITE IRC50

With AMBERLITE IRC50, the difficulties in the ion exchange process for basic amino acid separation can be eliminated. Treatment of the carboxylic acid exchanger with an appropriate buffer (sodium acetate-acetic acid) converts the resin into a combined salt-acid form so that cation exchange occurs at a controlled pH. If the ratio of sodium (salt form) to hydrogen (free-acid form) in the exchanger is adjusted to give pH below the isoelectric points of arginine, histidine and lysine, but above the isoelectric points of the neutral and acidic amino acids, only the basic amino will exist as cations in solution and will be adsorbed by AMBERLITE IRC50, while the other amino acids will pass through the resin bed unaffected.

Recovery of miscellaneous substances with AMBERLITE IRC50

The unique properties of AMBERLITE IRC50 have been exploited further by determining the adsorption-elution characteristics of the resin in the recovery of many ionic substances, including quinine, nicotine, thiamine, adenine, pyridine, hydrazine, ammonia, and sodium hydroxide. Both the hydrogen and sodium forms were studied in aqueous and alcohol solutions. The results of these investigations are reported in the following tables, classified bed.

RECOVERY OF MISCELLANEOUS SUBSTANCES

Substance Recovered	AMBERLITE IRC50 form	Solvent	Leakage %	Capacity g/l
Quinine sulphate	Na	H ₂ O	2	1320
Nicotine	Na	H ₂ O	100	—
Nicotine	H	H ₂ O	0-2	385
Thaimine hydrochloride	Na	H ₂ O	5-8	53.5
Thaimine hydrochloride	Na	C ₂ H ₅ OH	25	—
Adenine sulfate	Na	H ₂ O	100	—
Adenine	H	H ₂ O	100	—
Pyridine	H	H ₂ O	0	14
Hydrazine	H	H ₂ O	0-2	51.2
Ammonia	H	H ₂ O	0	78
Sodium hydroxide	H	C ₂ H ₅ OH	0	13

LIMITS OF USE

Rohm and Haas manufactures special resins for food processing and potable water applications. As governmental regulations vary from country to country, it is recommended that potential users seek advice from their Amberlite representative in order to determine the best resin choice and optimum operating conditions.

MATERIAL SAFETY DATA SHEETS

Material Safety Data Sheets (MSDS) are available for all Amberlite polymeric adsorbents. These

sheets contain pertinent information that you may need to protect your employees and customers against any known health or safety hazards associated with our products.

We recommend that you obtain copies of our MSDS from your local Rohm and Haas technical representative before using our products in your facilities. We also suggest that you contact your suppliers of other materials recommended for use with our products for appropriate health and safety precautions before using them.

All our products are produced in ISO 9002 certified manufacturing facilities.

Rohm and Haas/Ion Exchange Resins-Philadelphia, PA-Tel. (800) RHAMBER-Fax: (215) 537-4157
Rohm and Haas/Ion Exchange Resins-75579 Paris Cedex 12-Tel. (33) 140025000-Fax: 143452819

WEBSITE: <http://www.rohmhaas.com/ionexchange>



AMBERLITE is a trademark of Rohm and Haas Company, Philadelphia, U.S.A.

Ion exchange resins and polymeric adsorbents, as produced, contain by-products resulting from the manufacturing process. The user must determine the extent to which organic by-products must be removed for any particular use and establish techniques to assure that the appropriate level of purity is achieved for that use. The user must ensure compliance with all prudent safety standards and regulatory requirements governing the application. Except where specifically otherwise stated, Rohm and Haas Company does not recommend its ion exchange resins or polymeric adsorbents, as supplied, as being suitable or appropriately pure for any particular use. Consult your Rohm and Haas technical representative for further information. Acidic and basic regenerant solutions are corrosive and should be handled in a manner that will prevent eye and skin contact. Nitric acid and other strong oxidizing agents can cause explosive type reactions when mixed with ion exchange resins. Proper design of process equipment to prevent rapid buildup of pressure is necessary if use of an oxidizing agent such as nitric acid is contemplated. Before using strong oxidizing agents in contact with ion exchange resins, consult our sales knowledgeable in the handling of these materials.

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